0022-202X/80/7405-344$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 74:344–348, 1980

Vol. 74, No. 5
Printed in U.S.A.

Basophil Mediators and Their Release, with Emphasis on BK-A

HAROLD H. NEWBALL, M.D., HENRY L. MEIER, PH.D., AND LAWRENCE M. LICHTENSTEIN, M.D.

Pulmonary and Clinical Immunology Divisions, Department of Medicine, The Johns Hopkins University School of Medicine, at The Good Samaritan Hospital, Baltimore, Maryland, U.S.A.

During IgE-mediated events, basophils secrete small molecular weight mediators (histamine, slow reacting substance, Eosinophil chemotactic factor) which are thought to participate in inflammatory processes. We here describe the IgE-mediated secretion of large molecular weight mediators which have the potential for the activation of the Hageman factor dependent pathways, and the generation of biologically active peptides. These large molecular weight basophil derived mediators may, through the activation of the Hageman factor dependent pathways, influence mechanisms which participate in both acute and chronic cell-mediated inflammatory processes.

We suggest that these proteases may participate not only as mediators of the immediate hypersensitivity reaction, but may also function in important aspects of the entire inflammatory response.

Human peripheral leukocyte preparations from allergic donors, when challenged with the appropriate antigens, release several chemical mediators including histamine, slow reacting substance (SRS), and eosinophil chemotactic factors [1]. The release processes which are involved have been extensively described. In each instance, the mediators are actively secreted by a process which is temperature and calcium-dependent, require metabolic energy, and is controlled by hormone-receptor interactions which influence the intracellular level of cyclic nucleotides [2,3]. While much attention has been given to the possible role of these small molecular weight mediators in inflammatory or allergic reactions, little data has been available pertaining to the possible role of high molecular weight mediators which are also actively secreted during the IgE-mediated response. Recent studies of mast cell systems have revealed that, during secretory events, large molecular weight complexes may be secreted which possess biologically active molecules. Thus, heparin, bound to a large molecular weight proteoglycan, is secreted and is biologically active [4]. Our work, in the basophil system, also suggests that biologically active molecules are secreted bound to large molecular weight complexes (possibly fragments of granular matrices). We have described a high molecular weight complex which has at least 3 activities associated with it: a tosyl arginine methyl ester (TAMe) esterase activity, a kinin-cleaving protease, and a Hageman factor cleaving protease [5–9]. In this discussion, we will address ourselves primarily to the newly described large molecular weight mediators which we have termed "basophil kallikrein-like activities" (BK-A) and only briefly review new information regarding the small molecular weight mediators.

Supported in part by Grant HL 14153 from the National Heart, Lung and Blood Institute and Grant AI 07290 from the National Institute of Allergy and Infectious Diseases, The National Institutes of Health.

Publication No. 378 from the O'Neill Laboratories, The Good Samaritan Hospital.

Reprint requests to: Harold H. Newball, Department of Medicine, The Johns Hopkins University School of Medicine at the Good Samaritan Hospital, Baltimore, MD 21239.

Abbreviations:
AA: arachidonic acid
BK-A: basophil kallikrein-like activities
ECF: eosinophil chemotactic factors
HF: Hageman factor
NSAID: nonsteroidal anti-inflammatory drugs
PAF: platelet activating factor
SRS: slow reacting substance
TAMe: tosyl arginine methyl ester

Experimental Studies of the Basophil Kallikrein-like Activities

When basophil preparations are challenged with either purified protein antigens, such as ragweed antigen E, or with highly specific anti-IgE, they release a TAMe esterase activity. Our studies on the mechanism of the release of BK-A have primarily concentrated on this esterase activity since its measurement is relatively easy and precise. As will be indicated below, however, it cannot be assumed that TAMe esterase activity parallels each of the biological activities of BK-A.

A typical dose-response curve for the release of TAMe esterase is shown in Fig 1*A*. In general, the pattern of histamine and TAMe esterase release is similar, whether the release is initiated by antigen or by anti-IgE. The maximal percentage of histamine or TAMe esterase release has, however, no fixed relationship. This differential release of histamine and TAMe esterase is similar to the observations of others relating to differential release of enzymes from the neutrophil [10]. In this cell type, which shares a common cell of origin with the basophil, the release of beta-glucuronidase from the azurophil granule and lysosyme from the specific granule are often quite different. There have also been reports showing differential release of histamine and SRS-A from mast cells [11].

While the quantity of histamine and TAMe esterase release may differ, the rate of release of the 2 mediators shows no significant difference (Fig 1*B*). Similarly, release of both histamine and TAMe esterase is temperature and calcium-dependent. Not only is the initiation of release of TAMe esterase temperature sensitive but decreasing the temperature at any time during the release process stops the reaction abruptly. The release is also absolutely dependent upon the extracellular calcium concentration and the addition of EDTA at any time during the process causes a similar rapid cessation of release [7].

Modulation of TAMe esterase release has been studied using the same series of agonists utilized to explore the control of histamine release. Agonists which act on specific receptors to activate adenylate cyclase inhibit the 2 processes similarly. For example, Fig 2*A* shows that prostaglandin E_1 inhibits both processes in a dose-dependent fashion. Similar experiments have been carried out with beta-adrenergic agonists, histamine, and cholera enterotoxin. In each case, the inhibition dose-response curves were essentially identical. Agents, such as theophylline, which act by inhibiting the cAMP phosphodiesterase inhibit the release of histamine and of esterase with a similar pattern (Fig 2*B*). Cochicine, which causes disaggregation of microtubules, is a potent inhibitor of histamine release while heavy water (D_2O), which favors microtubular aggregation, is a potent enhancer of this process. Together, the 2 agents are antagonistic, so that their effects cancel. Similar studies with respect to esterase release showed that cochicine inhibited the process while D_2O caused a potentiation of release [7]. The

need for metabolic processes in the release of TAMe esterase was studied with 2-deoxyglucose, an inhibitor of glucose phosphorylation. These studies revealed that the TAMe esterase release is entirely energy-dependent. The concentration of 2-deoxyglucose required for 50% inhibition of release is similar for the TAMe esterase (0.4 mM) and histamine release (0.3 mM) [7].

After demonstrating that the release process and the pharmacologic control of esterase release was similar to that of the previously described mediators, we next attempted to purify the esterase and to ascertain its relationship to the biological activities (cleavage of kininogen to produce immunoreactive kinin and cleavage of Hageman factor) which had been observed in the crude supernatants. For this purpose, large quantities of the basophil supernatant were obtained by challenge of leukocyte preparations with anti-IgE or antigen E and these supernatants were sequentially chromatographed on Sephadex G-200, DEAE-Sephacel and Sepharose 6B. The chromatography on Sephadex G-200 yielded only one TAMe esterase-active peak, which was eluted in the void volume. When this was chromatographed on DEAE-Sephacel and eluted with a linear salt gradient, a single peak of esterase activity was again obtained. However, chromatography of this peak on Sepharose 6B led to a more complex pattern (Fig 3). A major peak of esterase activity was observed which coincided with the first absorption peak and eluted with an estimated molecular weight of 1.2 million. A second esterase peak was present, but was quantitatively of smaller magnitude and smaller molecular weight (c. 400,000). A third arginine esterase peak was variably present and was quantitatively of even smaller magnitude than the 2 other forms. This third form also had the smallest molecular weight, approximately 70,000.

These fractions were then examined for their ability to generate immunoreactive kinin and to cleave Hageman factor.

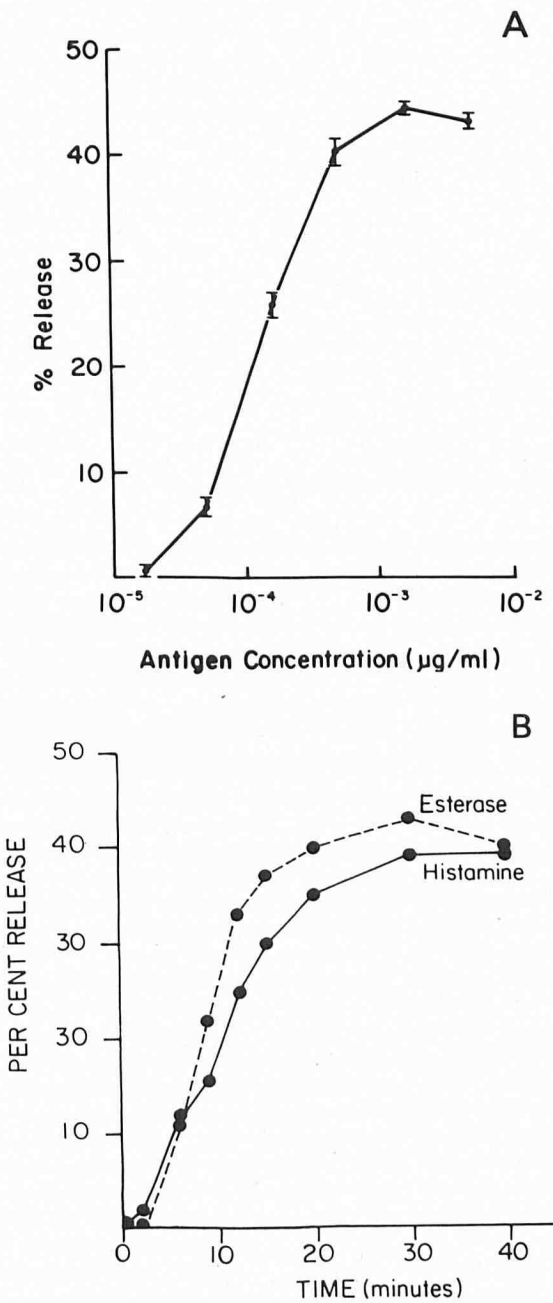

FIG 1. *a*, Dose-response of Rye Group I antigen induced release of a basophil TAMe esterase. *b*, Kinetics of AgE-induced (17 ng/ml) histamine and TAMe esterase release from basophils.

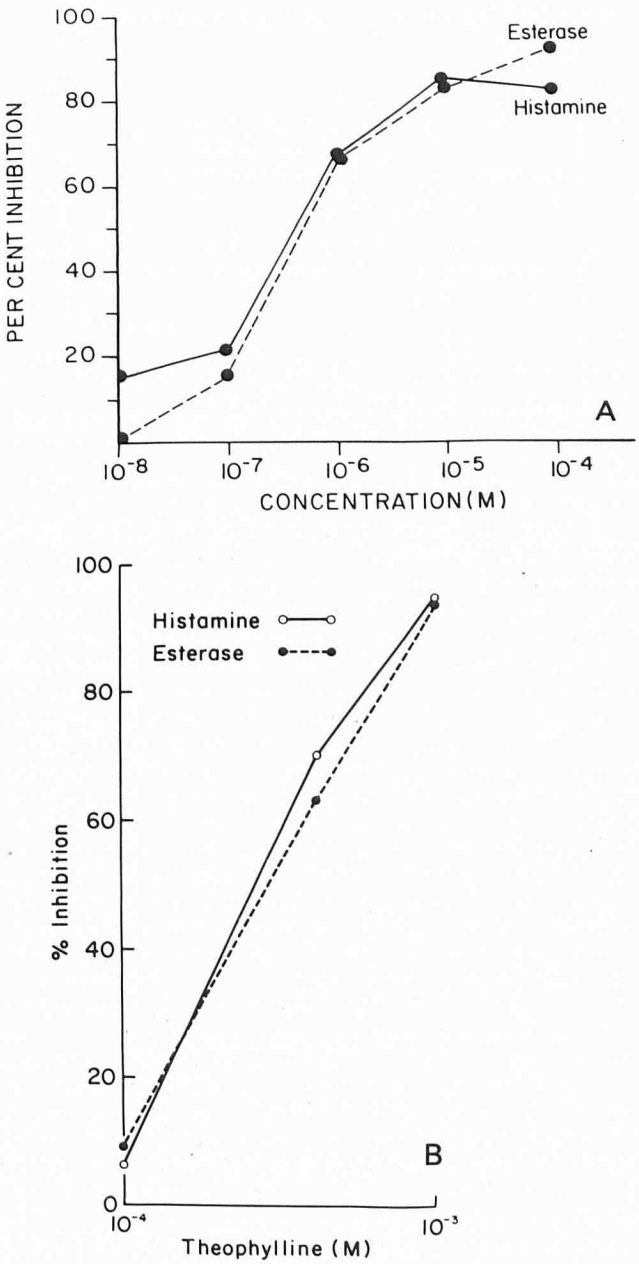

FIG 2. Inhibitory effects of PGE_1 (Fig 2*A*) and theophylline (Fig 2*B*) on TAMe esterase and histamine release from basophils. For each drug, the molar concentrations required for 50% inhibition of release are similar for histamine and the TAMe esterase.

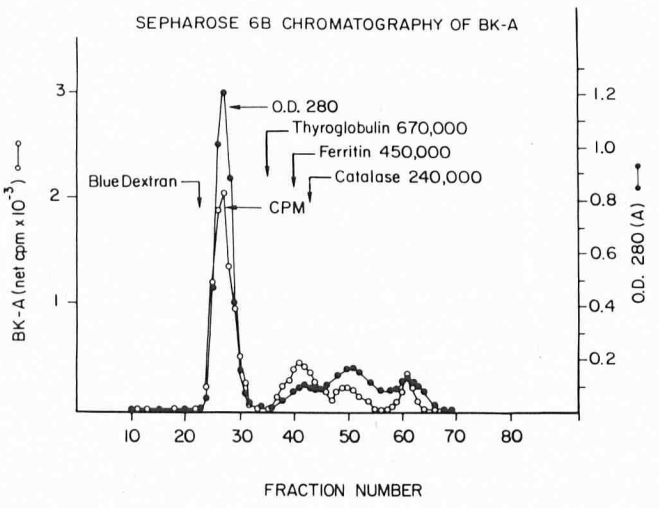

FIG 3. Sepharose 6B chromatography of TAMe esterase active fractions from DEAE-Sephacel.

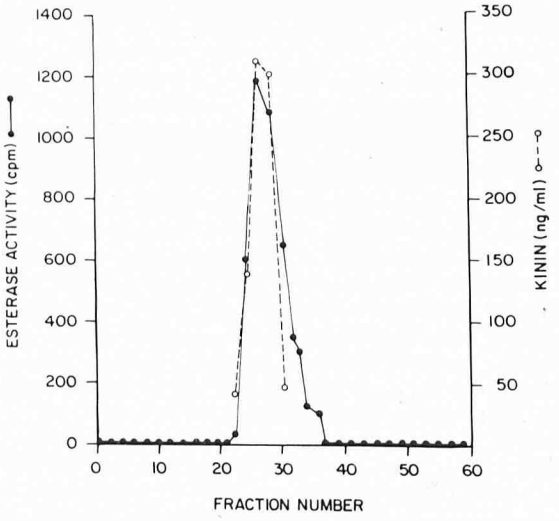

FIG 4. Arginine esterase activity and kinin generation by anti-IgE generated supernate chromatographed on Sepharose 6B (cpm × 10^{-1}).

Figure 4 shows the relationship between TAMe esterase activity and the generation of immunoreactive kinin from the first, high molecular weight peak of the Sepharose 6B column. It is evident that the 2 activities co-chromatograph. This assay was carried out with acid treated, heat inactivated plasma. More recently, it has been shown that the same protease can cleave purified, high molecular weight kininogen to yield immunoreactive kinin [6–7].

Similar studies have not been completed with respect to the mediator which cleaves Hageman factor. It is clear, however, that while there is TAMe esterase activity associated with the first 2 peaks, there is little present in the last, small molecular weight peak; fractions from the last peak were, however, most potent in cleaving Hageman factor.

The activity of the basophil Hageman factor (HF) protease is shown in Fig 5. Radiolabeled Hageman factor was adsorbed to a glass surface after which it was cleaved by either plasma kallikrein or the basophil HF protease. After cleavage, samples were analyzed by electrophoresis on SDS-PAGE as previously described [9,12]. The top panel shows intact Hageman factor, with a molecular weight of 80,000. The middle panel shows the cleavage of Hageman factor by purified human plasma kallikrein. Part of the radiolabeled Hageman factor remains intact while the rest is cleaved into fragments of approximately 28,000 and 52,000 daltons. The lower panel shows cleavage of Hageman factor by the basophil HF protease. As with plasma kallikrein, part of the radiolabeled Hageman factor remains uncleaved while the rest is split into fragments of 28,000 and 52,000 daltons. Additional studies have determined that the 28,000 molecular weight fragment produced by the basophil enzyme has functional activity as assessed by its ability to activate prekallikrein to kallikrein [9].

Studies with Other Mediators

Perhaps the most interesting recent observation with respect to several of the mediators has to do with the role of arachidonic acid (AA) metabolism in the release process. With respect to histamine release, it has been shown that ETYA, which blocks both the lipoxygenase and cyclo-oxygenase pathways of AA metabolism, completely inhibits the release of histamine. On the other hand, the nonsteroidal anti-inflammatory drugs (NSAID) such as indomethacin, aspirin and meclofenamic acid, which are selective inhibitors of the cyclo-oxygenase pathway of arachidonic acid metabolism, potentiate the release of histamine. These observations suggest that a product of the lipoxygenase pathway is necessary for histamine release and that the NSAID act by shifting arachidonic acid metabolism to that pathway. In keeping with this suggestion was the fact that arachidonic acid itself also potentiated histamine release [13]. A related observation was that indomethacin and other NSAID selectively block the inhibition of histamine release caused by each of the agonists (isoproterenol, histamine, adenosine, prostaglandins) which act through specific receptors to increase adenylate cyclase activity. Arachidonic acid has a similar activity. In contrast, neither indomethacin nor arachidonic acid had any effect on the inhibition of release caused by drugs or agents which increase cyclic AMP by other mechanisms. Thus, they did not reverse the inhibition caused by theophylline or isobutylmethylxanthine which are phosphodiesterase inhibitors; similarly, they had little or no effect on the inhibition caused by dibutyryl cyclic AMP itself [13]. These data strongly suggest that there must be subcompartments of cyclic AMP which are differently affected by cyclase agonists and by other agents

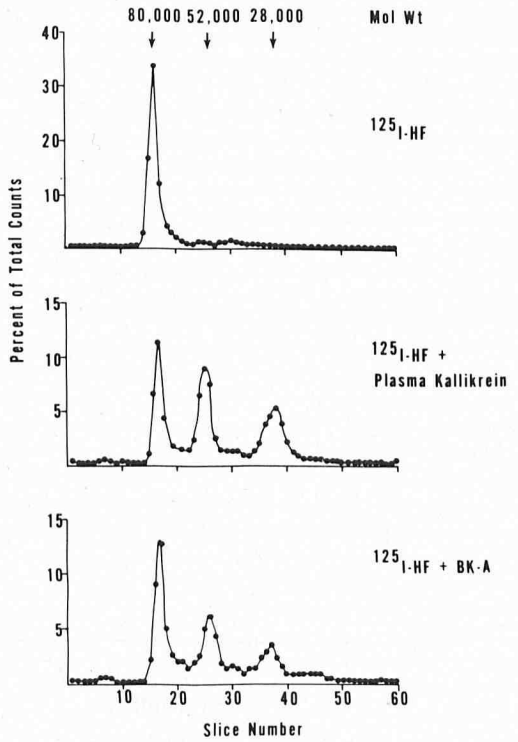

FIG 5. Cleavage of human Hageman Factor by preparations of the basophil HF protease.

which increase cyclic AMP. It further suggests that not only is there a product of the lipoxygenase pathway necessary for histamine release but that another (or the same) product of that pathway modulates the effects of endogenous hormones or pharmacologic agents on the control of histamine release.

Also related to arachidonic acid metabolism is the recently described structure of slow reacting substance (SRS). Samuelson et al have demonstrated that SRS is derived from the lipoxygenase pathway of arachidonic acid metabolism, with an additional step which incorporates cysteine into the molecule to yield what has been designated leukotriene C [14]. The precise role of SRS in inflammatory conditions is not fully elucidated but, with respect to smooth muscle, the duration of its action is known to be much longer than that of histamine. The definition of the structure of SRS should, for the first time, allow the synthesis of a series of antagonists which can delineate the role of this mediator in inflammatory processes.

Another interesting observation is that the major source of SRS in inflammatory reactions is probably not basophils or mast cells, but other cell types. This has been described by Bach in rat peritoneal cells [15] and we have demonstrated that SRS is readily derived from human polymorphonuclear leukocytes [16].

There are a number of eosinophil chemotactic factors (ECF) produced from mast cells and basophils. Two of these have been characterized as tetrapeptides by Goetzl and Austen [17]. However, here, as well, there is the recent observation that eosinophil chemotactic factors can be readily obtained from polymorphonuclear leukocytes by challenge with calcium ionophore or as a result of a phagocytic stimulus. There is further evidence that arachidonic acid is either the precursor of the neutrophil ECF or enhances its production [18]. Platelet activating factor (PAF) has been described in rabbits as a basophil mediator which causes the secretion of serotonin from platelets. In primate species, however, this lipid mediator also appears to be derived primarily from nonmast cells or basophil sources [19].

It is possible to generalize, then, that the nonpreformed lipid mediators (SRS, ECF, PAF) which have previously been associated with IgE-mediated reactions in mast cells or basophils exist in and are commonly released from other cell types. It is likely that these mediators are generated as a result of the increase in phospholipid metabolism which occurs in activated cells, not only during the allergic response, but in other cell types activated by appropriate stimuli.

DISCUSSION

We have tried to point out that, in addition to the small molecular weight mediators of inflammatory reactions, there is a series of high molecular weight mediators which are released from basophils following interaction of antigen and IgE antibody. These mediators have the ability to generate kinin from human plasma kininogen and to activate human Hageman factor. The importance of these mediators is illustrated in Fig 6. Thus, BK-A, by acting on Hageman factor, is capable of yielding the biologically active 28,000 molecular weight fragment B-HF_a which is capable of activating prekallikrein to kallikrein, which can either cleave plasminogen to produce plasmin or act on kininogen to generate kinin. Each of these activation steps is subject to control so that BK-A's additional ability to generate immunoreactive kinin from kininogen is an important "feed forward" aspect of these reactions. These are the first mediators from basophils (though similar mediators are released through IgE-mediated mechanisms in the mast cell [20] which are capable of interacting with the coagulation and kallikrein-kinin systems to generate biologically relevant peptides. In fact, this represents the only known immunologic pathway into these plasma cascade systems.

We have previously suggested that certain of the chemical mediators, such as histamine, participate not only in immediate-type inflammatory reactions but also function in important aspects of the entire inflammatory process. While the role of the basophil kallikrein-like activities in immediate hypersensitivity reactions and in other inflammatory processes is not clear, they do represent a first and important interface between IgE-mediated reactions and the Hageman factor dependent pathways of the inflammatory response. By virtue of these activities, the basophil kallikrein-like activities may influence mechanisms which are involved in both subacute and chronic, cell-mediated inflammatory processes.

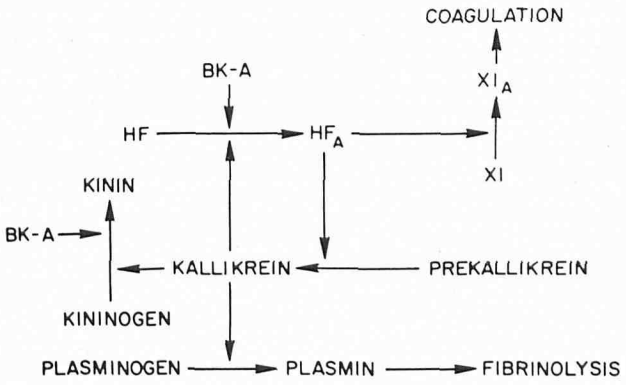

FIG 6. Activation of the Hageman factor dependent pathways of coagulation, fibrinolysis and kinin formation by a basophil HF protease.

There has been recent interest in the involvement of IgE-mediated mechanisms in delayed reactions and in cutaneous basophil hypersensitivity, both of which represent subacute or chronic phases of inflammation. Dvorak and his associates have shown that, in these types of reactions, there is activation of the coagulation system [21]. Perhaps, during an IgE-mediated skin reaction, basophils and mast cells secrete the above-described proteases which activate the Hageman factor dependent pathways, thus resulting in the inflammatory processes which are observed during delayed skin reactions. Studies by Dvorak and his associates and by Askenase also show the deposition of fibrin in and around inflammatory lesions associated with cutaneous basophil hypersensitivity [22–23]. It is possible that the basophils, through the mechanisms described above, activate the coagulation system and are responsible for the fibrin deposition. In addition to being involved in subacute and chronic inflammatory reactions, the mediators described here may be involved in more acute reactions. Thus, we have reported the consumption of coagulation factors during human anaphylaxis [24] and Pinckard and his associates have observed the same phenomenon in systemic anaphylaxis in rabbits [25]. There has been clinical evidence that there are coagulation abnormalities in anaphylactic reactions and BK-A may well provide the mechanism by which this occurs.

The basic biochemistry of the coagulation system and the kinin generating system has been appreciated for a considerable period of time. How this is activated *in vivo* has not been clear, particularly from an immunologic point of view and the mediators we have described represent the first link between antigen activated reactions and the plasma cascade systems.

The authors acknowledge that much of the work herein reported was carried out in collaboration with Dr. Richard Talamo, Johns Hopkins University School of Medicine, Baltimore, Md., Dr. Charles G. Cochrane and Susan Revak of the Research Institute of Scripps Clinic, La Jolla, CA. We thank Ms. Judy Mason for her technical assistance.

REFERENCES

1. Lichtenstein LM: Mechanisms of allergic inflammation, Progress in Immunology III, Edited by TE Mandel et al, North Holland Publishing, Amsterdam, 1977, pp 430–438
2. Lichtenstein LM, Margolis S: Histamine release in vitro: Inhibition by catecholamines and methylxanthines. Science 161:902–903, 1968

3. Lichtenstein LM: The immediate allergic response: In vitro separation of antigen activation, decay and histamine release. J Immunol 107:1122–1130, 1971
4. Metcalfe DD, Lewis RA, Silbert JE, Rosenberg RD, Wasserman SI, Austen KF: Isolation, identification, and characterization of heparin from human lung. J Allerg Clin Immunol 63:191, 1979
5. Newball HH, Talamo RC, Lichtenstein LM: Release of leukocyte kallikrein mediated by IgE. Nature 254:635–636, 1975
6. Newball HH, Berninger RW, Talamo RC, Lichtenstein LM: Anaphylactic release of a basophil kallikrein-like activity. I. Purification and characterization. J Clin Invest 64:457–465, 1979
7. Newball HH, Talamo RC, Lichtenstein LM: Anaphylactic release of a basophil kallikrein-like activity. II. A mediator of immediate hypersensitivity reactions. J Clin Invest 64:466–475, 1979
8. Newball HH, Revak SD, Cochrane CG, Griffin JH, Lichtenstein LM: Cleavage of Hageman Factor (HF) by a basophil kallikrein of anaphylaxis (BK-A). Clin Res 26:519A, 1978
9. Newball HH, Revak SD, Cochrane CG, Griffin JH, Lichtenstein LM: Activation of human Hageman Factor by a leukocytic protease, Kinins II, Part B, Plenum Publishing Co, New York, 1979, pp 139–151
10. Wright DG, Bralove DA, Gallin JI: The differential mobilization of human neutrophil granules: Effects of phorbol myristate acetate and ionophore A23187. Am J Pathol 87:273–283, 1977
11. Orange RP, Langer H: Proceedings of VIII International Congress of Allergology. Tokyo, Excerpta Medica, Amsterdam, 1973, p 325
12. Revak SD, Cochrane CG, Bouma BN, Griffin JH: Surface and fluid phase activities of two forms of activated Hageman factor produced during contact activation of plasma. J Exp Med 147:719–729, 1978
13. Marone G, Sobotka AK, Lichtenstein LM: Effects of arachidonic acid and its metabolites on antigen-induced histamine release from human basophils in vitro. J Immunol 123:1669–1677, 1979
14. Samuelsson B, Borgeat P, Hammarstrom S, Murphy RC: Introduction of a nomenclature: Leukotrienes. Prostaglandins 17:785–787, 1979
15. Bach MK, Brashler JR: Ionophore A 23187-induced production of slow reacting substance of anaphylaxis (SRS-A) by rat peritoneal cells in vitro: Evidence for production by mononuclear cells. J Immunol 120:998–1005, 1978
16. Conroy MC, Orange RP, Lichtenstein LM: Release of slow reacting substance of anaphylaxis (SRS-A) from human leukocytes by the calcium ionophore A23187. J Immunol 116:1677–1681, 1976
17. Goetzl EJ, Austen FK: Purification and synthesis of eosinophilotactic tetrapeptides of human lung tissue: Identification as eosinophil chemotactic factor of anaphylaxis. Proc Nat Acad Sci USA 72:4123–4127, 1975
18. Tesch H, Konig W, Frickhofen N: Eosinophil chemotactic factor, release from human polymorphonuclear neutrophils by arachidonic acid. Int Arch Allergy Appl Immunol 58:436–446, 1979
19. Clark PO, Hanahan DJ, Pinckard RN: Physicochemical identity of platelet activating factor (PAF) isolated from human neutrophils and monocytes and rabbit neutrophils and basophils. Fed Proc 38:6252, 1979
20. Meier HL, Newball HH, Berninger RW, Talamo RC, Lichtenstein LM: Purification of lung kallikrein of anaphylaxis. J Allerg 63: 191, 1979
21. DeShazo RD, Levinson AI, Dvorak HF, Davis RW: The late phase skin reaction: Evidence for activation of the coagulation system in an IgE-dependent reaction in man. J Immunol 122:692–698, 1979
22. Dvorak HF, Dvorak AM: Cutaneous basophil hypersensitivity, Progress in Immunology II, vol 3. Edited by L Brent, J Holborow. Amsterdam, North Holland Publishing Company, 1974, pp 171–181
23. Askenase PS: The role of basophils, mast cells and vasomines in hypersensitivity reactions with a delayed time course, Progress in Allergy, Edited by P Kallos, BH Waksman, Basel, S. Krager, 1977, pp 199–320
24. Kaplan AP, Hunt KJ, Sobotka AK, Smith P, Horakova Z, Gralnick H, Lichtenstein LM: Human anaphylaxis: A study of mediator systems. Clin Res 25:361A, 1977
25. Pinckard RN, Tanigawa C, Halonen M: IgE-induced blood coagulation alterations in the rabbit: Consumption of coagulation factors XII, XI, and IX in vivo. J Immunol 115:525–532, 1975

DISCUSSION

STREILEIN: Do you know anything about the differential release of BKA versus histamine?

LICHTENSTEIN: Yes, histamine and the esterase have the same dose response relationships and the same kinetics, but the absolute amounts which are released are quite different. It is almost as if there are 2 separate granules, one of which releases histamine and one of which releases this other mediator.

WUEPPER: Which of the 2 kininogens is the substrate for BKA?

LICHTENSTEIN: It has been demonstrated to cleave the high molecular weight kininogen; recently Kaplan said that it may also cleave the low molecular weight kininogen.

WUEPPER: Can you reduce that very high molecular weight material in any manner?

LICHTENSTEIN: I did point out that the chromatogram has 2 forms of the enzyme at 400,000 and 70,000 molecular weight; both have esterase activity. I think it's becoming clear now that the material which activates Hageman factor is not necessarily the same as that material which has kallikrein activity, but this is work in progress.

WUEPPER: Is the material performed in granule?

LICHTENSTEIN: The esterase is performed.

GIGLI: If you add the purified esterase to normal human serum, do you see any of the changes in serum proteins seen in the patient whom you described?

LICHTENSTEIN: Our work has been primarily involved in trying to purify the various activities. I do know whether addition of some of the peaks to plasma produces coagulation abnormalities, but they certainly have not been studied in any defined fashion.

MANNIK: Do you think the interaction of IgE molecules with receptor and the release phenomena are related primarily due to conformational changes of the antibody molecule vs. polyvalency of the ligands? Have association constants been compared between monomeric ligands and di- or polyvalent ligands? Finally, how does the Fc fragment of IgE interact with the receptors on mast cells?

LICHTENSTEIN: Terry Ishizaka has made an antireceptor antibody to rat mast cells and found that you do not need IgE to induce histamine release. All you have to do is bring 2 receptors together, so I think that IgE is, in a sense, irrelevant.

MANNIK: Then how do you explain the differences that you noted in terms of the inhibitors interfering differently, if you used an antigen vs. aggregates of IgE?

LICHTENSTEIN: With BPO_2, you get a very symmetrical dose response curve. It goes up, peaks, and returns to base line; you can relate the release mathematically to the number of IgE cross links. Anti IgE does almost the same thing. There are reasons to believe that anti-IgE stimulates release by cross-linking on 2 IgE molecules. Antigens almost surely form more complex interactions with the IgE. You can visualize clumps of antigen-antibody complexes on the cell surface. I believe that it is this which constitutes the different stimulus.

DVORAK: Do human basophils release proteases other than kallikrein during antigen induced degranulation?

LICHTENSTEIN: There are at least 2, and probably more activities coming out.

CLAMAN: I did not understand a point about the free receptors for IgE. If, indeed, the affinity of the receptors for IgE is so high, what determines what cells will have receptors for IgE which are occupied.

LICHTENSTEIN: It follows form very simple physical chemistry. If you measure the affinity of the IgE receptors for IgE in different individuals; they vary over about a 30-fold range, so that there is some diversity of receptor affinity. The only times that there are free receptors is there are low (20–30 ng/ml) concentrations of serum IgE. If you look at the equilibrium constant and the concentration of IgE you get as much saturation as you would expect.